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OPTIMIZATION AND CHARACTERIZATION OF LIPASE-CATALYZED SYNTHESIS OF PALM AMINO ACID SURFACTANT

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OPTIMIZATION AND CHARACTERIZATION OF LIPASE-CATALYZED SYNTHESIS OF PALM AMINO ACID SURFACTANT

By

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OPTIMIZATION AND CHARACTERIZATION OF LIPASE-CATALYZED SYNTHESIS OF PALM AMINO ACID SURFACTANT

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April 2009

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Optimization and characterization of lipase-catalyzed synthesis of palm amino acid surfactant was studied in 500 mL stirred tank reactor (STR). The reaction of palm kernel olein (PKO) and L(+)-lysine with Lipozyme *RM* IM as biocatalyst was optimized by response surface methodology (RSM). The study was divided into five parts which are; the optimization of reaction synthesis, the reactor study in term of mixing efficiency, the stability of immobilized enzyme, the purification process and the analysis and characterization of palm amino acid surfactant. A two level (2^4) full factorial central composite rotatable design (CCRD) was successfully employed for the experimental design and analysis of the results. The combined effect of temperature (X_1); (40.0-70.0°C), impeller speed (X_2); (100.0-400.0 rpm), substrate molar ratio (X_3); (1.0-4.0 mmol) and amount of enzyme (X_4); (5.0-8.0 g) was investigated. The optimum condition derived via RSM at fixed reaction time of 24 h was successfully optimized at

temperature; 47.50°C, impeller speed; 324.00 rpm, substrate ratio; 3.25 mmol and amount of enzyme; 7.25 g. The actual experimental yield was 89.03% under the optimum condition, which compared well with the maximum predicted value of 93.77%.

Reactor study on the performance of 2 L STR as a mixing device was evaluated to improve the mixing efficiency. The reaction was scaled-up to 360X with a total volume of 1.125 L. The rheological property of the reaction mixture exhibited Newtonian behaviour. Rushton turbine impeller showed better performance in degree of mixing, whereby a high Reynold number for a range between 10^2 and 10^4 was achieved from 100-400 rpm, which exhibited a transitional flow pattern as compared to AL hydrofoil impeller. The Lipozyme *RM* IM was shown to be quite stable based on its synthetic activity where the percentage yield of palm amino acid surfactant was decreased only after 4 recycles in STR. The effect of shear forces due to the mechanical impeller speed on the enzyme morphology was determined by scanning electron microscope (SEM). The percentage yield of palm amino acid surfactant was found to be low for Rushton turbine impeller and AL hydrofoil impeller with speed of 400 rpm and 200-400 rpm, respectively.

Purification process was successfully studied using liquid-liquid extraction technique, whereby high purity, 96.97% of palm amino acid surfactant was obtained. Analysis of the surfactant was determined by Fourier transform-infrared spectroscopy (FT-IR) and gas chromatography-flame ionization detector (GC-FID) to verify the identity and purity of the product isolated. Characteristics of palm amino acid surfactant were also examined, which include saponification value, acid value, iodine value and ester value.

Compatibility of the surfactant in most oils and its stability even after heating up to 90°C and overnight storage at room temperature showed that the surfactant has good potential to be used for further applications.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi syarat bagi mendapatkan ijazah Sarjana Sains

PENGOPTIMUMAN DAN PENCIRIAN SINTESIS SURFAKTAN ASID AMINO MINYAK KELAPA SAWIT MENGGUNAKAN PEMANGKIN LIPASE

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Pengoptimuman dan pencirian sintesis surfaktan asid amino minyak kelapa sawit telah dikaji menggunakan 500 mL reaktor tangki bergerak (STR). Tindak-balas menggunakan olein isirong kelapa sawit (PKO) dan L(+)-lisin dengan Lipozyme *RM* IM sebagai biopemangkinan telah dioptimumkan oleh kaedah permukaan respon (RSM). Kajian ini telah dibahagikan kepada lima bahagian iaitu pengoptimuman bagi sintesis tindak-balas, kajian reaktor berhubung dengan kecekapan percampuran, kestabilan enzim tersekat-gerak, proses penulenan dan analisis dan pencirian bagi surfaktan asid amino minyak kelapa sawit. Faktorial penuh pembolehubah kuasa dua (2^4) rekaan pusat komposit berputar (CCRD) telah berjaya digunakan sebagai rekabentuk eksperiman dan analisis untuk keputusan. Penggabungan kesan bagi suhu (X_1); (40.0-70.0°C), kelajuan putaran (X_2; (100.0-400.0 rpm), nisbah molar substrak (X_3); (1.0-4.0 mmol) dan jumlah enzim (X_4); (5.0-8.0 g) telah dikaji. Keadaan optimum yang dirumuskan melalui kaedah

RSM pada masa tetap 24 h, telah berjaya dioptimumkan pada suhu; 47.50ºC, kelajuan putaran; 324.00 rpm, nisbah molar substrak; 3.25 mmol dan jumlah enzim; 7.25 g. Nilai sebenar hasil eksperimen ialah 89.03% di bawah keadaan optimum, di mana ia telah dibandingkan sesuai dengan nilai anggaran maksimum iaitu 93.77%.

Kajian reaktor ke atas prestasi 2 L STR sebagai peralatan percampuran telah dinilai untuk meningkatkan kecekapan percampuran. Tindak-balas telah ditingkatkan skalanya kepada 360X dengan jumlah keseluruhan isipadu pada 1.125 L. Sifat reologi campuran tindakbalas telah menunjukkan sifat Newtonian. Penggerak turbin Rushton telah menunjukkan prestasi terbaik di dalam darjah percampuran, berbanding dengan penggerak AL hidrofoil, yang mana nombor Reynolds yang tinggi di antara julat 10^2 dan 10^4 telah dicapai dari 100-400 rpm, yang menunjukkan gerakan perantaraan bagi aliran mendatar dan aliran bergelora. Lipozyme *RM* IM telah menunjukkan kestabilan yang agak tinggi berdasarkan kepada aktiviti sintesisnya dengan peratus hasil surfaktan asid amino minyak kelapa sawit hanya berkurang selepas 4 kali penggunaan semula di dalam STR. Kesan tekanan daripada kelajuan putaran mekanikal terhadap morfologi enzim telah ditentukan dengan pengimbas mikroskop elektron (SEM). Peratus hasil surfaktan asid amino minyak kelapa sawit telah didapati rendah bagi penggerak turbin Rushton dan AL hydrofoil dengan kelajuan masing-masing pada 400 rpm dan 200-400 rpm.

Proses penulenan telah berjaya dilaksanakan menggunakan teknik pengekstrakan cecair-cecair, dengan ketulenan yang tinggi, 96.97% bagi surfaktan asid amino minyak kelapa sawit telah diperolehi. Analisis surfaktan ini telah ditentukan dengan kaedah spektroskopi inframerah (FT-IR) dan spektroskopi pengesan pembakaran ion-

kromatografi gas (GC-FID) untuk pengesahan identiti dan ketulenan produk yang telah diasingkan. Ciri-ciri surfaktan asid amino minyak kelapa sawit juga telah dikenalpasti, termasuk nilai saponifikasi, nilai asid, nilai iodin and nilai ester. Kesesuaian surfaktan ini di dalam kebanyakan minyak dan kestabilannya walaupun selepas dipanaskan sehingga 90°C dan disimpan semalaman pada suhu bilik telah menunjukkan prestasi terbaik untuk digunakan pada kegunaan di masa hadapan.

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or currently submitted for any other degree at UPM or other institutions.

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LIST OF ABBREVIATIONS

%	percentage
°C	degree celsius
μ	Viscosity
ANOVA	analysis of variance
CCRD	central composite rotatable design
cm	centimeter
cm^{-1}	reciprocal centimeter
et al.	and co-workers
FFA	free fatty acid
FT-IR	fourier transform-infrared
g	gram
GC-FID	gas chromatography-flame ionization detector
h	hour
L	liter
μL	microliter
mL	milliliter
mmol	milimole
N	normality
Pa	pascal
Pa.s	pascal second
P-value	probability value
PO	palm olein

PKO	palm kernel olein
R^2	determination of coefficient
Re	reynold number
rpm	rotation per minute
RSM	response surface methodology
RBD	refined, bleached and deodorised
sec	second
SEM	scanning electron microscope
STR	stirred tank reactor
TG	triglyceride
TLC	thin layer chromatography
v	volume
w	weight
HCl	hydrochloric acid
KI	potassium iodide
KOH	potassium hydroxide
$Na_2S_2O_3$	sodium thiosulphate
NaOH	sodium hydroxide

CHAPTER 1

INTRODUCTION

Amino acid surfactants are biodegradable, non-skin irritating and have minimal toxicity to the living body (Tabohashi *et al.,* 2000; Roosmalen *et al.*, 2004; Sanchez *et al.*, 2005). Apart from their excellent emulsifying characteristics, many acyl amino acids possess strong antimicrobial activity, which have gained importance in the fields of food additives, cosmetics and pharmaceutical products (Tyman, 1992; Sanchez *et al.,* 2005). The amphiphilic nature of these surfactants gives them unique properties contributing towards many applications in a broad range of industries. Observation by Kosaric (1993) led to a conclusion that surfactant is regarded as an essential facet in any industrial fields. For this reason, there is an urgent need to develop biodegradable and biocompatible surfactants with low toxicity and excellent emulsifying properties, particularly to compensate with increasing concerns about energy consumption, environmental and toxicological dangers.

Amino acid-based surfactants obtained from the combination of natural saturated fatty acids, alcohols and amines with different amino acid head group through ester and amide linkages are synthesized using chemical, enzymatic synthesis or both methodologies (Infante *et al.*, 2004). However, the utilization of homogenous chemical catalyst leads to several complexities such as the production of toxic catalysts, corrosion of equipments and excessive consumption of energy. Biodegradation contributes to accumulation of these hazardous compounds in the environment. Gunawan *et al.* (2004) reported that environmentally-friendly

enzymatic synthesis allows mild reaction conditions. Lipase catalyzing the hydrolysis of fats and oil is widely used as a biocatalyst in the production of amino acid surfactants (Reetz, 2002).

The production yield of hydrolase-catalyzed reactions is significantly dependent on the operation parameters such as reaction temperature, substrate concentration, enzyme concentration and solvent polarity where non-conventional media are used (Carrea & Riva, 2000). Conventional optimization method manipulates one variable parameter whilst other parameters are kept constant. Consequently, any interactions amongst these parameters are neglected whereas one apparent set of optimal conditions is achieved. However, Response Surface Methodology (RSM) was used in this study as the statistical method. RSM offers advantages in studying the effect of several variables - individually or in pairs of simultaneous and systematic variations of that variables. Therefore when RSM is applied, the number of experimental trials needed could be reduced (Rezzoug *et al.*, 2004).

The synthesis of N^{ε}-acyllysines using palm kernel olein and L(+)-lysine catalyzed by lipase was carried out in 500 mL stirred tank reactor (STR). Optimization study was carried out using RSM. The principle objective of this project is to optimize enzymatic process for the synthesis of amino acid surfactant using palm kernel olein (PKO) and L(+)-lysine as substrates. In achieving the main objective, the sub-objectives include;

I. To determine the optimum condition for the synthesis of N^{ε}-acyllysines using response surface methodology (RSM).

II. To study the effect of mixing on reactor performance using two type of impeller.

III. To study the stability and reusability of immobilized lipase based on the type of impeller used.

IV. To purify, identify and characterize the N^{ε}-acyllysines produced using optimized condition obtained.

CHAPTER 2

LITERATURE REVIEW

2.1 Surfactants

Surfactant is an abbreviation for surface active agent which literally means "active at surface". Surfaces involved could be between solid and liquid, air and liquid or between a liquid and a different immiscible liquid (Porter, 1994). Surfactants are amphiphatic molecules consisting of hydrophobic and hydrophilic moieties which are chemically linked to each other in a unique way (Sarney & Vulfson, 1995).

When a surfactant immerses in a solution, its concentration is higher at the surface than in the bulk of the liquid. This key property causes the surfactants to concentrate at the surface of the solution and from economically point of view this characteristic is crucial for food (Tyman, 1992), pharmaceutical (Benavides *et al.,* 2004) and cosmetics preparation (Sanchez *et al.*, 2005).

World wide production of surfactants in 2006 was around 12.5 M tonnes per year and is currently growing by about 500,000 tones per year. Around 60% of the surfactant production is used in household detergents, 30% in industrial and technical applications, 7% in industrial and institutional cleaning and 6% for personal care. In term of value, cationics are forecasted to account for 34% in year 2009. Non-ionics and anionics contribute to 22%, whilst silicones and amphoterics are respectively

12% and 7%. Edser (2006) also mentioned that fluro-surfactants would be 3% of the total use in 2009.

Some of their functional properties and applications are listed in Table 1.

Table 1. Surfactants-functional properties and applications (adapted from Mc Graw Hill, 1997)

Functional Properties	Applications
Emulsifications	Lotions, creams and food
Wetting	Detergent
Foaming	Bubble baths and toothpaste
Lubrication	Lubricating oils

2.2 Amino Acid Surfactants

From the environment conservation aspect point of view, amino acid-based surfactants are remarkable surfactants due to their biodegradable capability (Tabohashi *et al*., 2000), non-skin irritating nature (Roosmalen *et al.,* 2004) and have minimal toxicity effects towards living body (Sanchez *et al*., 2005). These surfactants are known to display excellent emulsifying properties and possess strong antimicrobial activities (Mhaskar *et al.,* 1992; Infante *et al.,* 2004), which are not fairly recognized in other readily available surfactants (Takehara, 1988). Furthermore, the productions of amino acid-based surfactants consume low-cost and renewable raw materials (Roosmalen *et al.,* 2004).